

Amendments to the Claims:

The following claims replace all prior versions and listings of claims in this application.

1. (Currently amended) A process for recovering refractile particles containing a heterologous polypeptide from bacterial periplasm in which the polypeptide is insoluble comprising:

(a) culturing bacterial cells, which cells comprise nucleic acid encoding phage lysozyme, nucleic acid encoding the heterologous polypeptide, a signal sequence for secretion of the heterologous polypeptide, and separate and different inducible promoters for each of the nucleic acid encoding the phage lysozyme and the nucleic acid encoding the heterologous polypeptide, ~~under conditions~~ whereby the heterologous polypeptide is secreted into the periplasm of the bacteria as an aggregate and the phage lysozyme accumulates in the cytoplasmic compartment, wherein expression of the nucleic acid encoding the phage lysozyme is induced by the addition of an inducer after about 50% or more of the heterologous polypeptide has accumulated;

(b) disrupting the cells mechanically to release the phage lysozyme so as to release refractile particles from cellular matrix; and

(c) recovering the released refractile particles from the periplasm, whereby chloroform is not used in any step of the process, and wherein the recovery step minimizes co-recovery of cellular debris with the released refractile particles.

2. (Previously presented) The process of claim 1 wherein the heterologous polypeptide is a mammalian polypeptide.

3. (Previously presented) The process of claim 2 wherein the mammalian polypeptide is a human polypeptide.

4. (Previously presented) The process of claim 3 wherein the human polypeptide is an insulin-like growth factor (IGF), DNase, or vascular endothelial growth factor (VEGF).

5. (Previously presented) The process of claim 4 wherein the human polypeptide is IGF-I.

6. (Previously presented) The process of claim 5 wherein the promoters for the phage lysozyme and polypeptide are, respectively, arabinose promoter and alkaline phosphatase promoter.

7. (Previously presented) The process of claim 6 wherein the inducer for arabinose is added in an amount of about 0-1% by weight.

8. (Previously presented) The process of claim 5 wherein the signal sequence is lamB.

9. (Previously presented) The process of claim 1 wherein the bacterial cells are Gram-negative cells.

10. (Previously presented) The process of claim 9 wherein the bacterial cells are E. coli.

11. (Previously presented) The process of claim 1 wherein the bacterial cells are transformed with one or two expression vectors containing the nucleic acid encoding the phage lysozyme and the nucleic acid encoding the heterologous polypeptide.

12. (Previously presented) The process of claim 11 wherein the bacterial cells are transformed with two vectors respectively containing the nucleic acid encoding the phage lysozyme and the nucleic acid encoding the heterologous polypeptide.

13. (Previously presented) The process of claim 11 wherein the nucleic acid encoding the phage lysozyme and the nucleic acid encoding the heterologous polypeptide are contained on one vector with which the bacterial cells are transformed.

14. (Canceled)

15. (Previously presented) The process of claim 1 wherein after disruption the cells are incubated for a time sufficient to release the heterologous polypeptide aggregate contained in the periplasm.

16. (Previously presented) The process of claim 1 wherein the recovery comprises sedimenting retractile particles containing the heterologous polypeptide.

17. (Previously presented) The process of claim 16 wherein the recovery takes place in the presence of an agent that disrupts the outer cell wall of the bacterial cells.

18. (Previously presented) The process of claim 17 wherein the agent is a chelating agent or zwitterion.

19. (Previously presented) The process of claim 18 wherein the agent is EDTA.

20. (Previously presented) The process of claim 16 wherein the sedimentation is by centrifugation and is at a relative centrifugal force of at least about 3000 x g.

21. (Previously presented) The process of claim 1 wherein the culturing step takes place under conditions of a cell density of about 40 to 150 g dry weight/liter.

22. (Previously presented) The process of claim 1 wherein the phage lysozyme is T4-lysozyme.

23. (Previously presented) The process of claim 1 wherein the culturing takes place at a scale of at least about 500 liters.

24. (Previously presented) The process of claim 1 wherein the bacterial cells are non-temperature-sensitive.

25. (Previously presented) The process of claim 1 wherein one or more of the nucleic acids, including the promoter therefor, is integrated into the genome of the bacterial cells.

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